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Influence of weather conditions on the quality of 'Ingrid Marie' apples and their susceptibility to grey mould infection

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ABSTRACT

Apple (*Malus domestica*) is one of the most popular fruits consumed around the world. Environmental factors influence the development and quality of apples. We determined the influence of weather conditions on the quality of 'Ingrid Marie' apples harvested from eight different orchards in south Sweden in the years 2015–2017 and their susceptibility to infection by grey mould (*Botrytis cinerea*). We infected apples and collected data on fruit firmness, starch index, weight of fruit and lesion size in addition to collecting data on temperature, rainfall, sunlight and humidity in the period April–September. High rainfall in early April, during tree flowering, and in early June, during early fruit development, correlated with improved quality, namely reduced lesion size and low firmness level. Furthermore, with humidity higher than 77% in early June apples became more tolerant to grey mould, while low temperatures and high humidity in a period from the end of August to end of September, during the end of the fruit cell enlargement stage, correlated with larger apples. We conclude that rainfall, humidity and temperature are important weather factors influencing the quality of apples and their susceptibility to grey mould. This information may help apple growers understand the effects of weather conditions on apples more in detail. From such updated information, preharvest techniques may be applied (e.g. pruning, nutrition, irrigation or drainage) to improve conditions and apple quality as well as to reduce their susceptibility to pathogen attack.

1. Introduction

Apples (*Malus domestica* Borkh.) is one of the most popular fruits and is consumed around the world as an excellent source of vitamins and minerals [1,2]. The global apple production reached 83.1 million tonnes in 2017, of which the highest share came from China with 41.4 million tonnes. The European Union contributed with 9.6 million tonnes and the USA with 5.2 million tonnes (https://en.wikipedia.org/wiki/List_of_countries_by_apple_production). Worldwide, apple producers are requested to produce high-quality apples for consumption as fresh fruit. Furthermore, apples should be tolerant to pathogen attack both at harvest and after long-term storage and postharvest transport [3–5]. Apples attract consumers by their appearance (colour, size and shape), texture, aroma, nutritional value, and by their taste, for example sweetness and acidity [6].

Apple production in Sweden covers about 1660 ha and the total annual yield was 27 000–28 000 tonnes in 2017 of which almost 90%

were cultivated in the province Scania, southernmost Sweden (the Swedish Agricultural Agency, October 24, 2018). Several main apple cultivars are produced in Sweden such as Ingrid Marie, Aroma, Jonagold, Gloster, Elstar, Alice and Katja [7]. The final quality of the apples is influenced by environmental factors such as light regime plus location and conditions in the orchard [2,8]; furthermore temperature, rainfall, humidity and orchard management all may influence apples [7]. These factors also affect the tolerance of fruit to infection and the development of pathogens [9]. Major losses of apple fruit during storage are caused by postharvest pathogens, such as *Botrytis cinerea* [10,11].

During the growth season, environmental and agronomic factors (such as temperature, light, orchard design, irrigation system and pruning) strongly affect the development and quality of apple fruits [2,12]. Different growing seasons and locations result in a high variability in fruit quality. Even different locations of trees in the same orchard and different positions of fruits within the same tree influence the quality [13].

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The necrotrophic pathogen *Botrytis cinerea* (teleomorph: *Botryotinia fuckeliana*) causes grey mould by its airborne spores and is infectious to more than 500 different species globally [14–16]. *B. cinerea* may infect after harvest through wounds and cause decay later during storage [15, 17,18]. Grey mould is one of the major diseases of apples worldwide and spreads during an extended period of storage, e.g. in bins or boxes and causing severe economic losses [19].

Most commercially grown apples are harvested before they are ripe and stored at low temperatures for several months in a controlled atmosphere [20]. Since apples have a high market value, the ability to maintain their long-term quality after harvest is of economic importance to growers [16].

The aim of the present study was to determine the influence of weather conditions on the quality of apple fruits and their susceptibility to infection by *B. cinerea*. We thus assessed the effects of preharvest weather conditions on the quality and tolerance of the fruits to post-harvest pathogens. To this purpose we collected weather data and determined properties of apples harvested from eight orchards in the province Scania in southernmost Sweden for three years (2015, 2016 and 2017, Fig. 1). We investigated fruit quality parameters and the development of disease in ‘Ingrid Marie’, a popular Swedish apple cultivar suffering a high susceptibility to grey mould infection (Fig. 2). Environmental data encompassing temperature, rainfall, sunlight and humidity were collected from local Davis weather stations, in the orchards or nearby. We based our work on the hypothesis that knowledge of the impact of preharvest weather conditions (such as temperature, sunlight, rainfall and soil humidity) on the quality of apples and the postharvest resistance of fruits to pathogen attack will allow apple growers to better adapt treatments and improve these parameters through other means. The results presented allowed us to scrutinize and determine which one of several weather factors that had the largest influence on the quality of apples during the growing season. Furthermore, we determined which

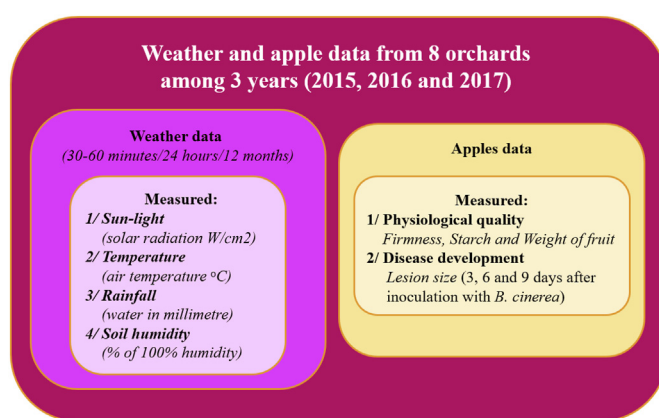


Fig. 2. Overview of the experimental setup (weather and apple data from eight orchards, among three years of harvest: 2015, 2016 and 2017). Weather data were collected encompassing temperature, rainfall, sunlight and humidity for each year. Apple data were collected encompassing lesion size (the development of grey mould), firmness, starch index and weight of the apples.

ones that reduced apple susceptibility to grey mould infection after harvest.

2. Materials and methods

2.1. Studied orchards

We used eight orchards located in Sweden's southernmost province, Scania. General Information about their locations is given below and in Fig. 1.

Orchard No.1 (55.43°N; 13.07°E), Orchard No.2 (55.48°N; 13.99°E), Orchard No.3 (55.66°N; 14.21°E), Orchard No.4 (55.71°N; 14.19°E), Orchard No.5 (55.72°N; 14.10°E), Orchard No.7 (56.16°N; 14.46°E), Orchard No.8 (55.72°N; 13.10°E) and Orchard No.9 (56.05°N; 12.75°E).

2.2. Harvested apple fruits

In each of the years 2015, 2016 and 2017, 150–180 fruits (cv. ‘Ingrid Marie’) were harvested from each of the orchards. Sampling dates were those of the regular time for harvest, ranging from end of September to mid-October, and dependent on the maturity of the fruits in each year and orchard as judged by Äppelrikt Österlen (an apple production company in the south of Sweden). The apples sampled in 2015 were immediately transported to the pcfuit, a research institute in Belgium, and those collected in 2016 and 2017 to the University of Gävle, Sweden. The experiments were carried out using the same design in both laboratories. The harvested apples were stored in a cold room (~2 °C) until the measurements started.

2.3. Experimental setup

In each of the years 2015, 2016 and 2017, we collected weather data (temperature, rainfall, sunlight and humidity) from the weather stations close to or in the 8 orchards, from the website of Fruit Web, Lammed, Sweden and the website of the Swedish Meteorological and Hydrological Institute (SMHI), and data from apples were harvested. Data for apples, namely weight of fruit, firmness level, starch index and lesion size of the fruits were collected at harvest and after storage for 1 and 3 months. The experimental setup is outlined in Fig. 2.

2.4. Data collection

2.4.1. Weather data

For all three years we collected weather data from 1st of January until 31st of December for each of the orchards. Because of the large amount of

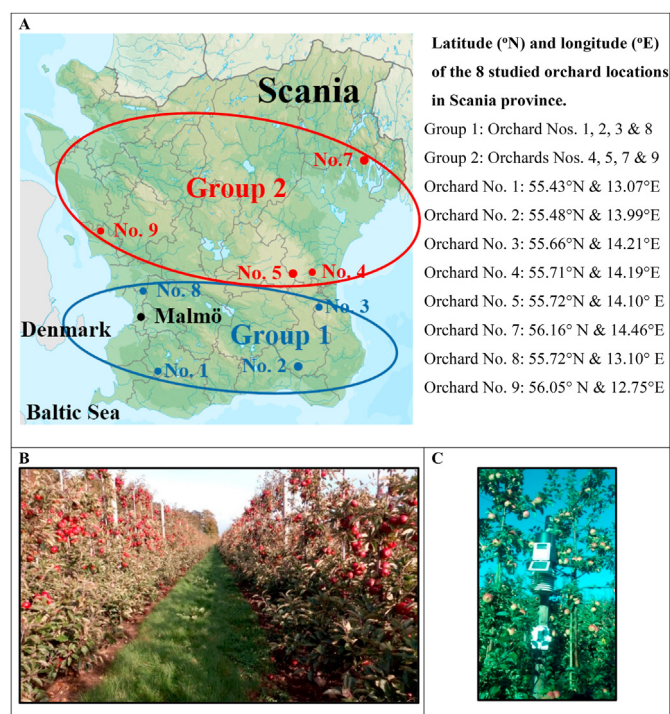


Fig. 1. The locations of eight orchard in the province Scania in southernmost Sweden (A). We grouped the orchards into two regional groups based on lesion size over the three years of harvest. In Group 1 lesion size varied less among the three years, whereas in Group 2 in the northern part of Scania the lesion size was much larger in the year of 2015 than in the years 2016 and 2017. ‘Ingrid Marie’ apple trees and fruits in the orchard (B). A Davis weather station in an apple orchard in southernmost Sweden (C).

weather data that needed to be collected and analysed we chose in the present study to focus on weather conditions during flowering, fruit growth and fruit development (according to Tromp and Wertheim [21] i.e. April–September).

Temperature ($^{\circ}\text{C}$), rainfall (mm) and humidity (%) were recorded automatically every 30 min using local Davis weather stations (Davis Vantage pro2 - wireless 6152, produced by Cole-Parmer, 625 East Bunker Ct Vernon Hills, IL 60061 USA) at each orchard and stored in the website of Fruit Web, Lammed, Sweden. Data for sunlight measured every 60 min was collected from the website of SMHI (W/cm^2).

We have used the term ‘temperature’ for mean weekly temperature (MT), and ‘humidity’ for mean weekly humidity (MH), ‘rainfall’ for sum weekly of rainfall (RS) and ‘sunlight’ for sum of weekly sunlight (SS).

2.4.2. Apple data

At the harvest and after each storage period (1 and 3 months), ten apples were used for determination of weight of individual fruits and of firmness level of two sides of fruit (the sun-exposed and shaded sides), starch index was determined for the whole fruit. These values were used as initial values for the apples to be incubated. Incubations were made using forty apples, of which thirty were inoculated with *B. cinerea*, and ten were control apples.

2.5. Inoculation of apples and evaluation of disease development

Spore suspensions of a frozen stock of *B. cinerea* were transferred to a fungal culture medium, i.e., potato dextrose agar. After two weeks, at the time for inoculation, *B. cinerea* spores were collected from the agar plates and a spore suspension of 1.5×10^5 spores per mL distilled water was prepared. Apples were wounded in the central part of each side using a sterile pipette tip and inoculated with a 20 μL spore suspension on the sun-exposed and the shaded sides. Following inoculation, the fruits were placed in plastic boxes covered by plastic foil and stored in a climate chamber at 15°C . The relative humidity was kept at 100% for the first 24 h, and then at 80% for the remainder of the experiment (for details see Bui et al. [11]).

Development of disease was followed quantitatively by measuring the diameter of lesions (cm) on the surface of inoculated apples using a plastic ruler. Lesion size was measured on both the sun-exposed and shaded sides of the fruits on days 3, 6 and 9 after inoculation with *B. cinerea*. We used the term ‘development of disease’ for lesion size.

2.6. Apples’ physiological determination

Firmness was estimated (kg/cm^2) (Tahir [7]), using a penetrometer (Bishop, fruit pressure tester, model FT327 - Italy) on the sun-exposed and the shaded sides. The determination was made by pressing the penetrometer at the middle point of each side, after removing a 0.5–0.7 cm diameter disc of peel (approximate depth 8 mm).

The starch-iodine test was used to estimate starch index (degradation stage) according to Tahir ([7]) with slight modifications. The iodine solution holds 40-g potassium iodide and 10-g iodine per L demineralized water. The solution is stirred for 3 h and then stored dark at 4°C until use. After dipping the slice of an apple into the iodine solution for 10 min, blue to black coloration gives an index of the starch contents. For cv. ‘Ingrid Marie’ we used a grading scale for starch index, which ranges from 1 to 10, where 1 = completely black and indicates maximum starch whereas 10 = completely white and indicates no starch.

Weight of fruit has been given in gram and measured as the average of the combined weight of 10 apples by using a balance showing two decimals.

2.7. Statistical analysis

Statistical analyses were performed in R 3.5.3 [22]. An over-all analysis of the data was made using a Principal Component Analysis

(PCA). The input to the ordination was weekly weather (mean weekly temperature and humidity, sum weekly of rain and sunlight) from April to September in eight orchards for three years (2015–2017). On this ordination lesion size, firmness level, weight of fruit and starch index in apples from the eight orchards and years was tested using the procedure *envfit* in the *vegan* package [23]. For lesion size, we combined the data from all the three different days after inoculation with *B. cinerea* (days 3, 6 and 9) and the data on two sides of fruit (sun-exposed and shaded). The next step was to explore for each quality variable which weather variables that had an influence. Since the number of observations (24) was small compared to number of explanatory variables (104) we used random forest as implemented in the wrapper algorithm in package Boruta [24] using the default selection criteria ($p < 0.01$). The selection of weather variables is based on confirmed importance from the Boruta algorithm; to find the direction of the association between weather variables and quality measurements we calculated the Pearson correlation coefficients. To explore possible interaction effects, we used the selected weather variables in regression trees using the package *rpart* [25].

3. Results

3.1. Weather conditions among the three years of harvest and among eight orchards

There were differences in temperature, rainfall, sunlight and humidity among the years 2015, 2016 and 2017 (Fig. 3). In general, in 2015 the weather was cold, dry, with low sunlight and low rainfall, in 2016 the weather was warm, dry, with high sunlight and heavy rainfall, while in 2017 the weather was cold, wet, with high sunlight and heavy rainfall, as well as high humidity (Fig. 3). Temperature in May–June (weeks 21–24) was lowest in 2015, somewhat higher in 2017 and highest in 2016

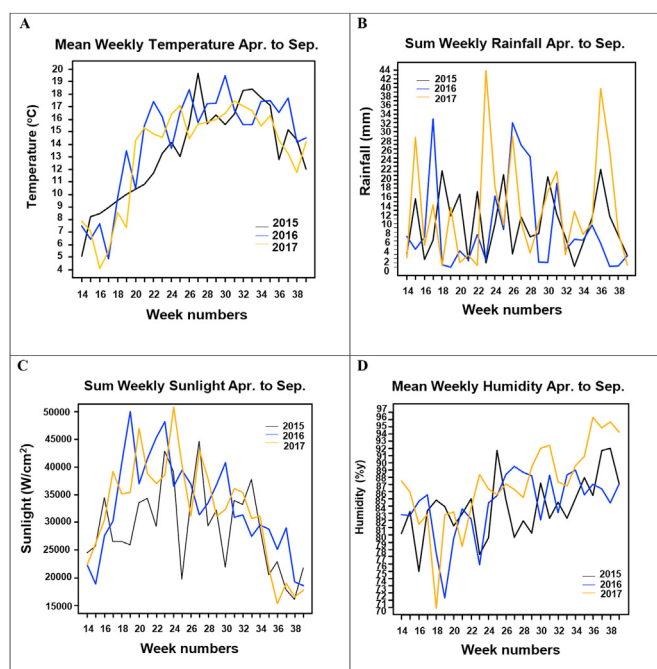


Fig. 3. Graphs of weather conditions from April to September (from week 14 - beginning of April until week 39 - end of September) for each of the three years of harvest (2015, 2016 and 2017). The mean weekly temperature from April to September among the three years is given as degree Celsius ($^{\circ}\text{C}$) (A). The mean weekly rainfall from April to September among the three years is presented in millimeters (mm) (B). The sum weekly of sunlight from April to September is given as unit Watts per square centimeter (W/cm^2) (C). The sum weekly of humidity from April to September is presented as % humidity (D).

(Fig. 3A). Rainfall was lowest in April and August (weeks 16, 33) in 2015, somewhat higher later in April and June (weeks 17, 28, 29), whereas in 2016 and in 2017 it was highest in April, June and September (weeks 15, 24, 37) (Fig. 3B). Sunlight was lowest in May, June and July (weeks 18–22, 25 and 30) in 2015, and somewhat higher in 2016 and 2017 (weeks 19–25) (Fig. 3C). In 2015 humidity was lowest in April (week 16), June (week 28–30) and July (week 34), somewhat higher in July for 2016 and highest in June, July and August in 2017. Humidity sank in early May (weeks 18–20) during both 2016 and 2017 (Fig. 3D).

Temperature, rainfall, sunlight and humidity in the period from April to September were different among the years for all 8 orchards. Temperature (April through September) was lowest in 2015 with 16 °C, somewhat higher in 2017 with 16.5 °C and highest in 2016 at 17 °C (Fig. 4A). Rainfall (April through September) was lowest in 2016 with 7 mm, higher in 2015 with 18 mm and highest in 2017 with 21 mm (Fig. 4B). Sunlight (April through September) was lowest in 2015 with 3500 W/cm², somewhat higher in 2017 at 3900 W/cm² and highest in 2017 at 4000 W/cm² (Fig. 4C). Humidity (April through September) was highest in 2017 at 92% and lower for both 2015 and 2016 at 88% (Fig. 4D).

There was no difference in weather conditions among orchards for the period from April to September for the three years (Fig. 5). The average weekly temperature from April through September all the three years and in all orchards was around 17 °C (Fig. 5A). That means that the orchards' locations had no influence on the temperature. Rainfall was not different for orchards Nos. 1, 4, 7, 8 and 9 with around 18–20 mm, somewhat lower for orchards Nos. 2 and 5 with 17 mm and lowest for orchards No. 3 with 9 mm (Fig. 5B). Sunlight showed no difference for all eight orchards and was around 3800 W/cm² (Fig. 5C). On a final note, humidity showed no difference for orchards Nos. 1, 2, 3, 4, 7, 8, and 9 at 89%, while it was higher for orchard No. 5 at 94%, (Fig. 5D).

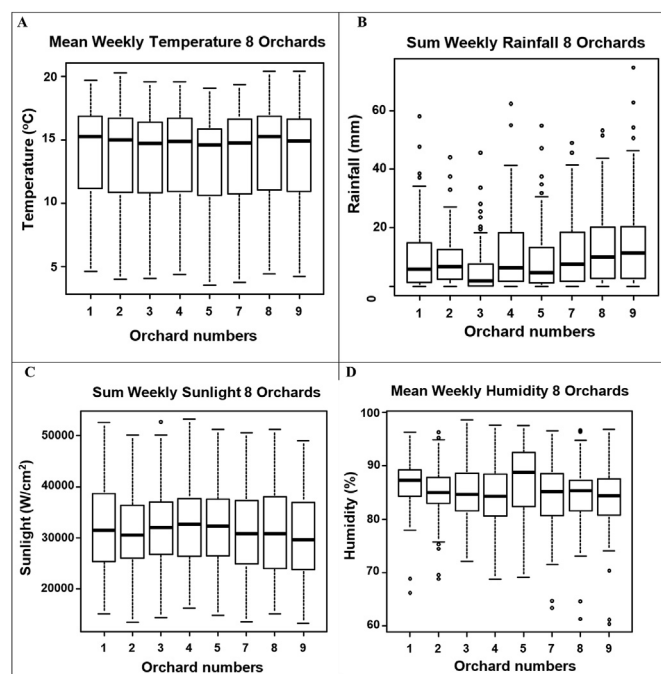


Fig. 5. Box plots presenting weather conditions for each of the 8 orchards (Nos 1, 2, 3, 4, 5, 7, 8 and 9) for the period from April to September for the three years of harvest (2015, 2016 and 2017). The mean weekly temperature of the eight orchards is presented in degrees Celsius (°C) (A). The mean weekly rainfall of the eight orchards is presented in millimeters (mm) (B). The sum weekly of sunlight for the eight orchards is given as unit of Watts per square centimeter (W/cm²) (C). The sum of weekly of humidity of the eight orchards is given as % humidity (D).

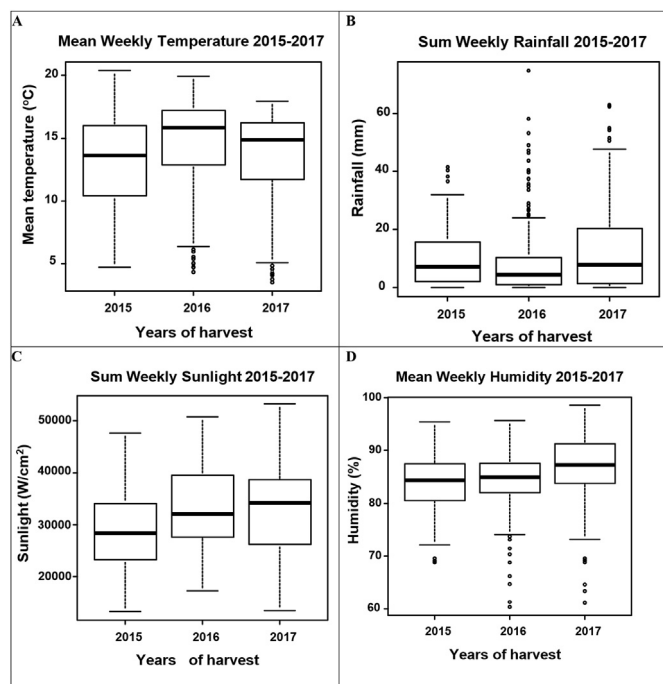


Fig. 4. Box plots presenting weather conditions of all 8 orchards for the period from April to September among the three years of harvest (2015, 2016 and 2017). The mean weekly temperature among the three years is given as degree Celsius (°C) (A). The mean weekly rainfall among 3 years is presented in millimeters (mm) (B). The sum weekly of sunlight among 3 years is presented in the unit of Watts per square centimeter (W/cm²) (C). The sum weekly of humidity among 3 years is presented as % humidity (D).

3.2. The influence of weather conditions on apple quality and disease development

An analysis of the data for weather vs apples' quality and susceptibility was made using a Principal Component Analysis (PCA) (Fig. 6). There were major differences in weather conditions and apple quality between the years, and small differences between orchards within a year (Fig. 6).

The harvest in 2017 produced high quality apples with the highest weight and the least susceptibility to *B. cinerea* infection. Year 2016 produced fruit with a high starch index and less susceptible to *B. cinerea* infection than in 2017, while 2015 produced the most susceptible apple fruits with the largest lesion size and the highest firmness (Fig. 6). Our results show that a high level of firmness was positively associated with the development of disease (Fig. 6). In contrast, high fruit weight and high starch index appeared to make apples more tolerant to infection.

We further analysed the results presented in Fig. 6 by calculating the correlation coefficients to find the direction of the association between weather factors and (i) quality of apples and (ii) lesion size. Humidity had the strongest influence, while rainfall and sunlight had less influence and temperature was least influential on the lesion size (Table 1). High humidity showed significant negative effects on lesion size on two occasions, namely in mid-May (week 20) with a correlation coefficient = −0.68 and in early June (week 23) with a correlation coefficient = −0.77. Humidity also displayed a significant negative interaction with the level of firmness on two occasions, namely in early June (week 23) at correlation coefficient = −0.76 and at the end of July-early August (week 31) at correlation coefficient = −0.75.

Conversely, there was a positive correlation (correlation coefficient +0.85) between humidity and firmness in early September (week 36). In addition, high humidity had a positive and significant effect on the weight of fruits during two periods, one in the end of July (week 30) with

Weather correlated to apple quality & fruit susceptibility (2015-2017)

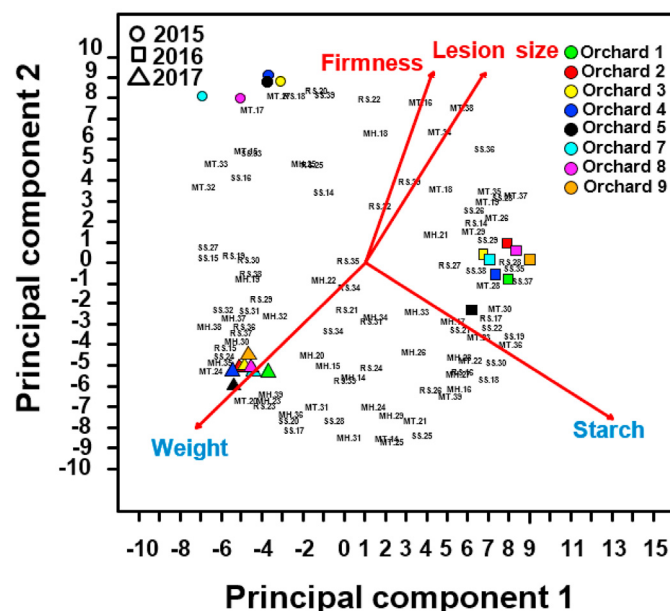


Fig. 6. A PCA of the association/correlation between weather conditions and apple quality and the development of disease among the three years of harvest (2015, 2016 and 2017) among eight studied orchards in the province of Scania. The weather conditions are given as mean weekly temperature-MT, mean weekly humidity-MH, sum weekly of rainfall-RS and sum weekly of sunlight-SS for April to September. Apple quality is given as weight of fruit, firmness and starch index and as development of disease (lesion size). The three years of harvest are shown in different forms, circle for 2015, square for 2016 and triangle for 2017. The eight orchards are indicated in different colors. Green, red, yellow, dark blue, black, blue, pink and dark yellow represent orchard Nos 1, 2, 3, 4, 5, 7, 8, and 9, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the correlation coefficient = +0.74 and finally in September (weeks 37–39) with correlation coefficients between +0.62 and +0.67 for all three weeks (Table 1).

Temperature had a strong and positive impact on firmness in no less than seven of the weeks, with the most pronounced effects in mid-April (week 16) at a correlation coefficient = +0.90, mid-September (week 38, +0.84), mid-June (week 24, +0.74) and mid-August (week 34, +0.74). In addition, high temperature during four weeks in a period from mid-August to mid-September (weeks 34, 35, 37 and 38) displayed negative effects on fruit weight (correlation coefficients between –0.80 and –0.67).

Weather conditions in mid-May (week 20) and early June (week 23) had a strong influence on apple quality characteristics, namely at the level of firmness, starch index and weight of fruit, and on fruit susceptibility to infection by grey mould (lesion size-disease development). As mentioned above, high humidity in the middle of May (week 20) negatively affected lesion size, whereas high temperature or a high sunlight in the same week had a negative impact on firmness (correlation coefficients = –0.87 and –0.89, respectively). Conversely, high rainfall in week 20 had a positive impact on firmness (correlation coefficient = +0.74).

High humidity in early June (week 23) negatively affected both lesion size and firmness (as mentioned above) and high rainfall during the same week had significant and negative effects on lesion size (correlation coefficient = –0.72) and firmness (correlation coefficient = –0.87, Table 1). On a final note, high temperature week 23 had a positive effect on the starch index (correlation coefficient = +0.67).

3.3. Different weather conditions impact differently on specific fruit quality parameters

The strong association of humidity with the protection against disease can also be seen in the regression tree for mean lesion size (Fig. 7A). A humidity higher than 77% in early June (week 23) produced tolerant apple fruits with the smallest lesion size; 52% of the apples had a mean lesion size of 2.0 cm as compared to the mean lesion size of 2.5 cm (Fig. 7A). When combined with low sunlight (<42 000 W/cm², week 23) the average lesion size was 1.8 cm, whereas, additionally, a humidity higher than 89% this week produced the most tolerant apples; 10% of the apples had a mean lesion size of 1.6 cm. In contrast, a humidity lower than 77% early June (week 23) and lower than 79% in the middle of May (week 20), as well as a rainfall lower than 8.9 mm in mid-June (week 24) resulted in the most susceptible apples with the largest lesion size (10% of the apples had a mean lesion size of 3.7 cm) (Fig. 7A).

The mean firmness of apples was strongly associated with rainfall in early June (week 23); at rainfall higher than 19 mm apples with low firmness were produced; 38% of the apples had a mean firmness of 2.6 kg/cm² as compared to a mean firmness of 3.7 kg/cm² (Fig. 7B). When combined with a temperature in mid-August (week 34) higher than 15 °C the highest quality apples with the lowest firmness were produced, namely 24% of the apples displaying a mean firmness value of 2.3 kg/cm². However, rainfall in early June (week 23) lower than 19 mm resulted in apples of a poor quality with high firmness (62% of the apples with a firmness of 4.4 kg/cm²). In addition, when the temperature in early May (week 19) was lower than 10 °C the apples displaying the highest firmness were produced with 19% of the apples displaying a mean firmness of 5.0 kg/cm².

The mean starch index of apples was also associated with rainfall and temperature (Fig. 7C). The apples with the highest starch index (best quality) were produced when the rainfall at the end of April (week 17) was higher than 11 mm and lower than 22 mm at the end of June (week 26, 19% of the apples with a starch index of 6.7). If, in addition, the temperature in early May (week 18) was lower than 9.9 °C, the highest starch index was recorded with 5% of the apples having a starch index of 7.6, compared to the mean starch index of 5.0. In contrast, a rainfall at the end of April (week 17) lower than 11 mm and a temperature in early June (week 23) lower than 13 °C produced poor apples with a low starch index, 10% of the apples had a mean starch index of 2.5.

The mean weight of apple fruits was strongly associated with temperature. A temperature lower than 17 °C at the end of August (week 35) produced heavy apples; 48% of the apples had a weight of 153 g as compared to a mean weight of 138 g. In addition, when the temperature was higher than 17 °C in mid-June (week 24) the heaviest apples (14% of the apples had an average weight of 164 g) were produced. In contrast, with the temperature higher than 17 °C at the end of August (week 35) and around 18 °C in mid-August (week 34), the smallest apples were produced (5% of the apples had a mean weight of 102 g). Moreover, temperatures higher than 18 °C in the latter half of August (weeks 34 and 35) also produced smaller fruit (19% of the apples had a mean weight of 120 g) (Fig. 7D).

4. Discussion

Our results are in agreement with those of several publications, which report that both environment and preharvest weather as well as conditions in the orchard affect fruit quality [2,13,26–29]. Iwanami [28], Minas et al. [4], and Musacchi and Serra [2] demonstrated that quality properties, such as firmness, starch index, sugar content, colour, weight and size have been found to vary widely among growing seasons. Other studies show that seasonal conditions affect fruit growth, which in turn is known to influence the quality of fruit [26]. In 2015, the weather was cold, dry, with low sunlight and low rainfall (Fig. 3), and apples were produced with a higher firmness and bigger lesion size, viz. a higher susceptibility to grey mould infection as compared to those harvested in

Table 1

The positive/negative correlation of weather conditions from April to September on the quality apples among the three years of harvest and among eight orchards. Weather conditions presented by mean weekly temperature-MT (°C), mean weekly humidity-MH (%), sum weekly of rainfall-RS (mm) and sum weekly of sunlight-SS (W/cm²) and quality of apple presented by firmness level (kg/cm²), starch index (10 scale), weight of fruit (gram) and lesion size (cm). Period time from April to September presented by week numbers (week 14 in early April to week 39 at the end of October), three years harvest were 2015, 2016 and 2017 and eight orchards were Nos. 1, 2, 3, 4, 5, 7, 8 and 9.

Variables	Lesion size (cm)		Firmness level (kg/cm ²)		Starch index (scale from 1 to 10)		Weight of fruit (gram)	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Mean Weekly Humidity (MH) (%)								
14				-0.62				
20		-0.68						
23		-0.77		-0.76				
30							+0.74	
31				-0.75				
36			+0.85					
37		-0.50		-0.49			+0.60	
38	+0.65			-0.54			+0.67	
39							+0.62	
Mean Weekly Temperature (MT) (°C)								
16			+0.90					
18					+0.48			-0.66
19			+0.55					-0.75
20				-0.87				
23					+0.67			
24			+0.74				+0.71	
26			+0.50					
34			+0.74					-0.80
35								-0.67
37			+0.60					-0.77
38			+0.84					-0.79
Sum Weekly of Rainfall (RS) (mm)								
15		-0.65		-0.65			+0.74	
17					+0.54			
20			+0.74					
23		-0.72		-0.87				
24		-0.53						
26					+0.27			
Sum Weekly of Sunlight (SS) (W/cm²)								
15						-0.69		
17				-0.88			+0.73	
18					+0.67			
20				-0.89				
23	+0.62							
24			+0.64			-0.46	+0.79	
31				-0.59				
36			+0.76					-0.74

2016 and 2017 (Fig. 6). In 2016 the weather was warm, dry, with high sunlight and heavy rainfall, which resulted in apples with a higher starch index and a lower susceptibility (Fig. 6). In 2017 the weather was cold, wet, with high sunlight and heavy rainfall as well as high humidity (Fig. 3), resulting in apples with the highest weight and tolerance to grey mould infection (Fig. 6). Our results suggest that higher firmness correlated with a larger lesion size for all the three years and for all orchards. This association within a cultivar is in agreement with several studies described below. Tahir [7] found that several apple cultivars with high firmness were more susceptible to infection by the fungi *Neofabrae* spp. and *Colletotrichum gloeosporicoides*. On a similar note, De Castro et al. [30] suggested that high firmness correlated with earlier stages of maturity at harvest time and a strongly decreased the storability and postharvest quality of apples.

Our results on high starch index and weight of apples showing a negative correlation with the development of disease are in the agreement with Davey et al. [31] and Doerflinger et al. [32]. Davey et al. [31] reported that apples which at harvest had a higher quality (bigger size, heavier fruit and higher levels of vitamin C and antioxidants) had an increased capacity to resist pathogen attack after harvest. Doerflinger et al. [32] demonstrated that starch index, one of the simplest and easiest tests to determine harvest time, is clearly affected by preharvest conditions. Starch hydrolysis begins at the end of the fruit development and the conversion of starch to sugar is one of the processes that most clearly indicate the ripening stage of apple. The maximum starch index in fruit at harvest is affected by cultivar, climate and position in the tree and a light cropping tree have higher starch index in the fruit as seen by darker colour.

4.1. Influence of specific weather conditions on fruit quality

A novel finding in the study is that rainfall, humidity and temperature were the most important weather factors, whereas sunlight was less important as a factor that influenced the quality of apples and their susceptibility to grey mould infection. These results are slightly at odds with the traditional view that rain is an unfavourable weather factor during fruit development and that sunlight is the most important factor. Accordingly, Tromp [8] reported that sunlight is important for flowering and in determining the size of apple fruits. Tromp and Wertheim [21] determined that light conditions in the orchard are important for fruit quality and development. Our results point to that especially high rainfall in the middle of April, high rainfall and high humidity in early June, and low temperature at the end of August strongly influenced the quality of apples and their susceptibility to grey mould infection (Table 1; Figs. 7 and 8).

The strong association of humidity with the protection against disease can also be seen in the regression tree for mean lesion size (Fig. 7A). A humidity higher than 77% in early June (week 23) produced tolerant apple fruits with the smallest lesion size (Fig. 7A). In contrast, a low humidity in early June (week 23) combined with low rainfall in mid-June (week 24) resulted in the most susceptible apples with the largest lesion size (Fig. 7A). The mean firmness of apples was strongly associated with rainfall in early June (week 23); at high rainfall apples with low firmness were produced (Fig. 7B). However, low rainfall in early June (week 23) resulted in apples of a poor quality exhibiting high firmness. The mean starch index of apples was also associated with rainfall and temperature (Fig. 7C). The apples with the highest starch index (best quality) were

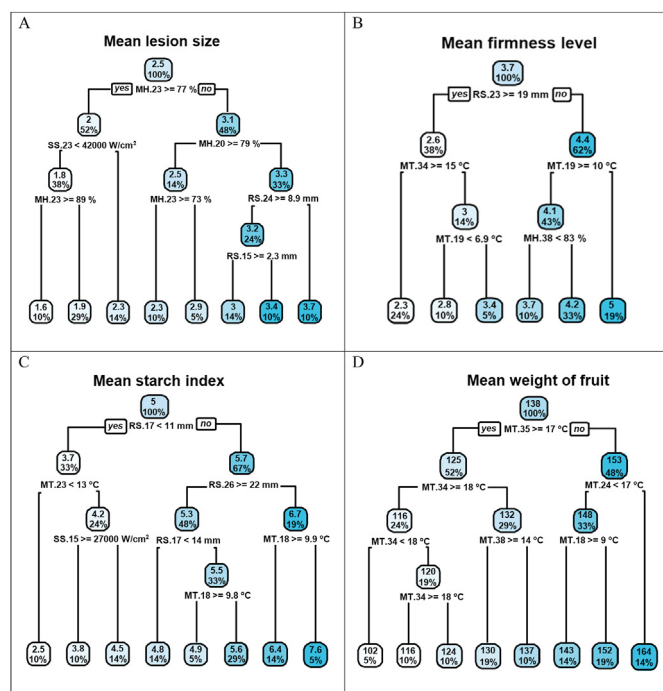


Fig. 7. Regression trees of the effect of weather conditions from April to September on specific apple quality parameters for the eight orchards and the three years of harvest. The weather conditions shown are mean weekly temperature-MT, mean weekly humidity-MH, sum weekly of rainfall-RS and sum weekly of sunlight-SS from week 14 - beginning of April to week 39 - end of September. Apple-quality measurements presented are weight of fruit, firmness, starch index and the development of disease (lesion size). Effects of weather on the mean lesion size (A), on the mean firmness (B), on the mean starch index (C) and on the mean weight of fruit (D).

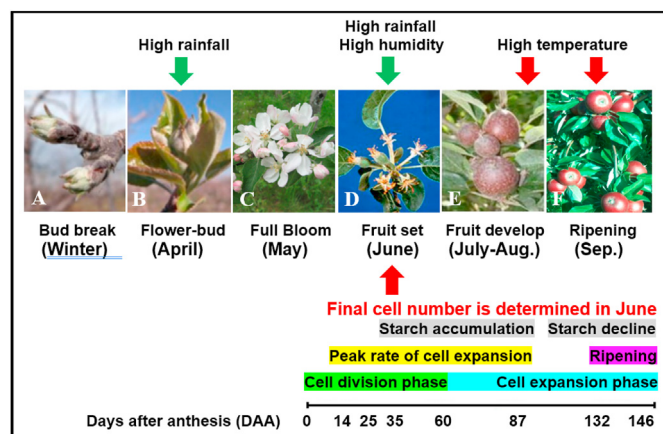


Fig. 8. The influence of preharvest weather (rainfall, humidity and temperature) during specific stages of growth and fruit development of apples. Bud break occurs during winter (November to March) (A), flower bud during April (B), full bloom during May (C), fruit set during June (D), fruit development during July–August (E) and ripening in September (F). The cell division phase occurs after full bloom (from May to June) and the cell enlargement phase occurs from fruit set to ripening (June–September).

produced when the rainfall at the end of April (week 17) was high and that in the end of June (week 26) was low. The mean weight of apple fruits was strongly associated with temperature. A low temperature at the end of August (week 35) produced heavy apples. Moreover, high temperatures in the latter half of August (weeks 34 and 35) also produced smaller fruit (Fig. 7D).

The flowering stages run from dormant buds in early winter to leaf fall in autumn. Apples bloom in April–May, petals fall in early June, fruit sets in July and fruit matures and ripens between 60 days after full bloom up to 180 days before harvest in September and October (Fig. 8). Poor weather conditions at the time of flowering, in particular frost damage, are major causes for poor fruit setting and yields in temperate climates [33].

In agreement with an importance of high temperature during flowering on fruit quality, we observed a negative impact of high sun and high temperature during apple flowering (week 20) on fruit firmness. In contrast, the trend is rather the opposite at earlier time-points, since we can see a strong positive effect of high temperature in April (week 16) on firmness. However, a positive effect of high rainfall during tree flowering (mid-April, week 15) that we observed (Table 1) may relate both to the importance of an adequate supply of water in this period as well as to the beneficial effect of rain spreading fertilizer administered.

Both high rainfall and humidity in early June (week 23) produced tolerant fruit (smallest firmness and lesion size, Fig. 7). At this stage flowering has ceased and early fruit development has started (Fig. 8). During flowering, cell-division activity in the ovary is very low and cell expansion does not occur [33]. After fertilization, a period of rapid cell division starts, which continues for 3–6 weeks in apple. Cell enlargement has already commenced at around the time of fertilization but increases when cell division has stopped. Cell number and cell volume together determine the ultimate fruit size. In agreement with the importance of weather conditions in mid-May (week 20) and early June (week 23) the first few weeks after full bloom (approximately during the period of cell division) have been pointed out as of paramount significance for fruit maturation at the end of the season [33]. One reason for this is that fruit set and early fruit growth is dependent on the production on hormones, mainly auxin and gibberilins, by the developing seeds, which exert control over the hormone balance within the whole tree.

Insufficient nutrition during this period may, due to large consequences on hormone balance in the entire tree, stall both seed and embryo development and lead to embryo abortion. In general, shoot growth is more sensitive to water stress than fruit growth but there is a strong dependency on the season [21]. Our data also stress the importance of a high-water supply during early fruit development for fruit quality. Our results indicate that low water supply (low humidity and rainfall) in early June (week 23) and low humidity and high temperature in September reduce fruit quality and makes the apples more susceptible to grey mould. This finding is opposite to that of [21], who reported that low relative humidity increases flower-bud formation and reduces shoot growth and that apple quality increases under water-stress conditions.

Furthermore, the positive effect of high humidity in September (weeks 37–39) on fruit weight and the strongly negative effect of high temperatures in mid-August to mid-September on the same parameter may also relate to water supply (Table 1). In pears, the sensitivity of fruit to water stress increases when drought occurs in the later season, in the period of rapid fruit growth (cell expansion), and drought in this period may reduce both fruit size and yield [21]. Similarly, in apples water has a higher influence on fruit size and on the quality in the second part of the season (the period of cell expansion). The access of the tree to water affects the weight of the fruit at harvest [33].

A positive effect of sunlight on weight and starch index of apple fruit as well as on sugar content and a negative effect of sunlight on firmness and acidity was recorded by keeping apple trees at different shade levels in the period from 8 weeks post bloom until harvest (from end of August, viz. at peak rate of cell expansion phase to September or October at fruit maturity). Our results suggest that high sunlight at the end of April (week 17) had a negative influence on firmness, whereas high sunlight in the beginning of May (week 18) positively influenced starch content. In agreement with previous data on the importance of sunlight, we found that high sunlight in mid-June (week 24) stimulated fruit weight (Table 1).

5. Conclusions

This paper is a first presentation of the influence of preharvest weather conditions among different years, and among orchards on the quality of apple fruits. Further, we report that the main weather factors, namely rainfall and humidity in early June (during early fruit development period) and at the end of August (fruit cell enlargement period) are strongly associated with fruit quality after harvest. A better understanding of the influence of weather factors on apple fruit quality may guide growers how to treat their apple trees and fruits based on the weather conditions a specific year in order to achieve improved fruit quality and a higher tolerance to pathogen attacks after harvest. Further studies on soil chemistry, dates of flowering and harvest as well as rootstock and nutrition in the orchards for at least 5 years are needed in order to understand more in detail how preharvest conditions influence apple quality and fruit susceptibility to pathogen attacks.

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